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RAW SEQUENCE LISTING DATE: 02/05/2002 PATENT APPLICATION: US/10/022,249 TIME: 14:19:13

Input Set : N:\jumbos\022249

Output Set: N:\CRF3\02052002\J022249.raw

ENTERED

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4 <110> APPLICANT: Vega, Manuel
             Drittanti, Lila
     7 <120> TITLE OF INVENTION: HIGH THROUGHPUT DIRECTED EVOLUTION BY RATIONAL MUTAGENESIS
     9 <130> FILE REFERENCE: 37851-911
C--> 11 <140> CURRENT APPLICATION NUMBER: US/10/022,249
C--> 12 <141> CURRENT FILING DATE: 2001-12-17
    14 <150> PRIOR APPLICATION NUMBER: 60/315,382
    15 <151> PRIOR FILING DATE: 2001-08-27
    17 <160> NUMBER OF SEQ ID NOS: 735
    19 <170> SOFTWARE: FastSEQ for Windows Version 4.0
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    22 <211> LENGTH: 621
    23 <212> TYPE: PRT
    24 <213> ORGANISM: Artificial Sequence
    26 <220> FEATURE:
    27 <223> OTHER INFORMATION: Mutant rep protein: rep78 4 GCT
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                                       25
    34 Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
           35
                                   40
    36 Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
    37
                               55
    38 Thr Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
                           70
                                               75
    40 Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Met His Val Leu Val Glu
                       85
                                           90
    42 Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
    43
                   100
                                       105
    44 Arg Glu Lys Leu Ile Gln Arg Ile Tyr Arg Gly Ile Glu Pro Thr Leu
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             115
                                   120
    46 Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly
    47
                               135
                                                   140
    48 Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
                           150
                                               155
    50 Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Gln Tyr Leu
                      165
                                           170
    52 Ser Ala Cys Leu Asn Leu Thr Glu Arg Lys Arg Leu Val Ala Gln His
                                      185
                  180
    54 Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Gln Asn
                                   200
         195
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	36-4		T	170 1	C1	Trp		1727	7 cr	T 110	C111		Пhr	Sar	Glu	Luc
		Giu	Leu	vai	GLY		Leu	vai	кър	шуз	235	116	T 11T	Der	Giu	240
	225		-1-	a 1	a 1	230	01 -	×1-	a	m		000	Dho	7.00	71-	
	GIn	Trp	TTE	GII		Asp	GIII	Ala	Ser		11e	Ser	Pile	ASII		Ala
61					245			_		250	_				255	Ŧ
62	Ser	Asn	Ser		Ser	Gln	тте	гàг		Ата	ьeu	Asp	Asn		GTA	гÀг
63				260	_		_	_	265		_	_		270		
64	Ile	Met	Ser	Leu	Thr	Lys	Thr		Pro	Asp	\mathtt{Tyr}	Leu		GLY	GIn	GIn
65			275					280					285			
66	Pro	Val	Glu	Asp	Ile	Ser	Ser	Asn	Arg	Ile	${ t Tyr}$	Lys	Ile	Leu	Glu	Leu
67		290					295					300				
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70	Thr	Lys	Lys	Phe	Gly	Lys	Arg	Asn	Thr	Ile	Trp	Leu	Phe	Gly	Pro	Ala
71		-	-		325					330					335	
72	Thr	Thr	Glv	Lvs	Thr	Asn	Ile	Ala	Glu	Ala	Ile	Ala	His	Thr	Val	Pro
73				340					345					350		
	Phe	Tvr	Glv		Va l	Asn	Trp	Thr	Asn	Glu	Asn	Phe	Pro	Phe	Asn	Asp
75		-1-	355	012				360					365			-
	Cve	Va1		Lvs	Met	Val	Tle		Trn	Glu	Glu	Glv		Met.	Thr	Ala
77	Cys	370	nsp	ц	1100		375					380	-1-			
	Lvc		Val	Glu	Sor	Ala		Δla	Tlo	T.e.11	Glv		Ser	Lvs	Va 1	Ara
	385	Val	val	GIU	261	390	μys	AIG	116	пец	395	OLY	DCI	1,5	• • •	400
		7 ~~	C1 n	T *** C	Crra	Lys	202	cor	7 l a	Cln		λen	Dro	ጥኮሎ	Dro	
	val	ASP	GIII	гух		гуѕ	Ser	ser	нта		TIE	кар	PIO	TILL	415	var
81	7		m1	a	405	m1	.	34-4	a	410	37a 1	т1.	N a m	C1		Cor
	TTE	vaı	Thr		Asn	Thr	ASI	мет		Ата	val	ше	ASD		ASII	ser
83		_,		420			a 1.		425	a1	3	3	34-4	430	T	Dha
	Thr	Thr		GIu	Hls	Gln	GIn		Leu	GIN	Asp	Arg		Pne	гаг	Pne
85	_		435			_	_	440	_	_,		_	445	1.		a 1 .
86	Glu		Thr	Arg	Arg	Leu		His	Asp	Phe	Gly		val	Tnr	Lys	GIn .
87		450			_		455		_	_		460				3
		Val	Lys	Asp	Phe	Phe	Arg	Trp	Ala	Lys		His	Val	Val	GLu	
	465					470					475					480
90	Glu	His	Glu	Phe	${ t Tyr}$	Val	Lys	Lys	Gly		Ala	Lys	Lys	Arg		Ala
91					485					490					495	_
92	Pro	Ser	Asp	Ala	Asp	Ile	Ser	Glu		Lys	Arg	Val	Arg		Ser	Val
93				500					505					510		
94	Ala	Gln	Pro	Ser	Thr	Ser	Asp	Ala	Glu	Ala	Ser	Ile	Asn	Tyr	Ala	Asp
95			515					520					525			
96	Arg	Tyr	Gln	Asn	Lys	Cys	Ser	Arg	His	Val	Gly	Met	Asn	Leu	Met	Leu
97	-	530					535					540				
98	Phe	Pro	Cys	Arg	Gln	Cys	Glu	Arg	Met	Asn	Gln	Asn	Ser	Asn	Ile	Cys
	545		-	_		550					555					560
		e Thi	r His	s Gl	v Glr	ı Lvs	Ası	Cys	Let	ı Glu	ı Cys	s Phe	e Pro	o Vai	l Se	r Glu
10:					565			- 4		570	ວ ໋				57	
		r Glr	n Pro	Va '			. Val	Lvs	s Lvs			c Glr	ı Lys	s Lei		s Tyr
103			\	580				1.	58		- 1 -		-1	590		4 -
		- Hic	s Hid			Gls	, Lv	s Va			o Ala	a Cv	s Th			s Asp
Ŧ0.	· TT	- 111.5	- 1112		- A1C1	- 01)		. · u.	'			- J.			7.	- -

RAW SEQUENCE LISTING
PATENT APPLICATION: US/10/022,249

DATE: 02/05/2002
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Input Set : N:\jumbos\022249

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105
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107 610
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121 20
122 Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
                             40
123 35
124 Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
                         55
126 Thr Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
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                     70
128 Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Met His Val Leu Val Glu
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                 85
130 Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
                     105
132 Arg Glu Lys Leu Ile Gln Arg Ile Tyr Arg Gly Ile Glu Pro Thr Leu
                                            125
                           120
133 115
134 Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly
135 130
                        135
136 Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
                     150
                                       155
138 Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Gln Tyr Leu
                                                    175
                                   170
                 165
140 Ser Ala Cys Leu Asn Leu Thr Glu Arg Lys Arg Leu Val Ala Gln His
                                185
141 180
142 Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Gln Asn
143 195
                             200
144 Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
                         215
                                           220
146 Met Glu Leu Val Gly Trp Leu Val Asp Lys Gly Ile Thr Ser Glu Lys
                     230
                                       235
147 225
148 Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
                                   250
                 245
150 Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
151 260
                                265
152 Ile Met Ser Leu Thr Lys Thr Ala Pro Asp Tyr Leu Val Gly Gln Gln
                         280
                                               285
153 275
154 Pro Val Glu Asp Ile Ser Ser Asn Arg Ile Tyr Lys Ile Leu Glu Leu
                        295
156 Asn Gly Tyr Asp Pro Gln Tyr Ala Ala Ser Val Phe Leu Gly Trp Ala
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PATENT APPLICATION: US/10/022,249

DATE: 02/05/2002
TIME: 14:19:13

Input Set : N:\jumbos\022249

```
157 305
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 158 Thr Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
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 160 Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Thr Val Pro
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                                   345
 162 Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
                               360
 164 Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
                           375
 166 Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
                        390
                                           395
 168 Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
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                                       410
 170 Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
 171
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                                   425
                                                      430
 172 Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
 173 435
                               440
 174 Glu Leu Thr Arg Arg Leu Asp His Asp Phe Gly Lys Val Thr Lys Gln
                          455
                                              460
 176 Glu Val Lys Asp Phe Phe Arg Trp Ala Lys Asp His Val Val Glu Val
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                                           475
 178 Glu His Glu Phe Tyr Val Lys Lys Gly Gly Ala Lys Lys Arg Pro Ala
                 485
                                       490
 180 Pro Ser Asp Ala Asp Ile Ser Glu Pro Lys Arg Val Arg Glu Ser Val
 181 500
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 200 Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
                               40
 202 Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
                           55
 204 Thr Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
                       70
                                          75
 206 Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Met His Val Leu Val Glu
                                      90
208 Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
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Input Set : N:\jumbos\022249

000				100					105					110		
209	_	-1	_	100	-1	a 1 .			105	•	01	-1.	a 1 .	110	ml	.
	Arg	GIU		ьeu	TTE	GIN	Arg	Ile	TYL	Arg	GTĂ,	TTE		Pro	Thr	Leu
211			115		- •	-		120		_	_		125		~ •	
	Pro		Trp	Phe	Ala	Val		Lys	Thr	Arg	Asn		Ala	GIA	GLY	GTA
213		130					135					140				
214	Asn	Lys	Val	Val	Asp		Cys	${\tt Tyr}$	Ile	Pro	Asn	Tyr	Leu	Leu	Pro	${ t Lys}$
	145					150					155					160
216	Thr	Gln	Pro	Glu	Leu	Gln	Trp	Ala	Trp	Thr	Asn	Met	Glu	Gln	Tyr	Leu
217					165					170					175	
218	Ser	Ala	Cys	Leu	Asn	Leu	Thr	Glu	Arg	Lys	Arg	Leu	Val	Ala	Gln	His
219			-	180					185					190		
220	Leu	Thr	His	Val	Ser	Gln	Thr	Gln	Glu	Gln	Asn	Lys	Glu	Asn	Gln	Asn
221			195					200				-	205			
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223		210		1			215		5		-1-	220			- 3	4 -
	Met		Len	Val	Glv	Trp		Val	Asp	Lvs	Glv		Thr	Ser	Glu	Lvs
	225	O.L.u	204	,	011	230	200			272	235				0-4	240
		Trn	τlΔ	Gln	Glu		G1n	Ala	Sor	Фυν		Ser	Dho	Δen	Δla	
227	GIII	115	116	GIII	245	пор	GIII	AIG	ber	250	110	DCI	1 110	ASII	255	AIG
	C ~ ~	200	Com	7 ~~		C1 n	т1.	Lys	777		LOU	Nan	N an	7.1 -		Tvc
		ASII	ser		Ser	GIII	TTE	цуs		нта	ьеи	ASP	ASII	270	GTA	цув
229		34-4	a	260	m l	T	m 15	» l -	265		m	T	77- 1		a1 -	C1 =
	TTG	мет		ьeu	THE	гăг	THE	Ala	Pro	Asp	TAT	Leu		GTÀ	GTII	GIII
231			275	_	_,	_	_	280	_	- 1	_	_	285	_	~1	_
	Pro		GLu	Asp	тте	ser		Asn	Arg	тте	Tyr		тте	Leu	GIU	Leu
233		290					295	_				300	_			
		Gly	Tyr	Asp	Pro		\mathtt{Tyr}	Ala	Ala	Ser		Phe	Leu	Gly	Trp	
	305					310					315					320
236	Thr	Lys	Lys	Phe	Gly	Lys	Arg	Asn	Thr	Ile	Trp	Leu	Phe	Gly	Pro	Ala
237					325					330					335	
238	Thr	Thr	Gly	Lys	Thr	Asn	Ile	Ala	Glu	Ala	Ile	Ala	His	Thr	Val	Pro
239				340					345					350		
240	Phe	Tyr	Gly	Cys	Val	Asn	Trp	Thr	Asn	Glu	Asn	Phe	Pro	Phe	Asn	Asp
241			355					360					365			
242	Cys	Val	Asp	Lys	Met	Val	Ile	Trp	Trp	Glu	Glu	Gly	Lys	Met	Thr	Ala
243	_	370	_	_			375		_			380				
244	Lys	Val	Val	Glu	Ser	Ala	Lys	Ala	Ile	Leu	Gly	Gly	Ser	Lys	Val	Arg
	385					390	-				395	_		-		400
		Asp	Gln	Lvs	Cvs	Lvs	Ser	Ser	Ala	Gln	Ile	Asp	Pro	Thr	Pro	Val
247			,	-1-	405	4				410					415	
	Tle	Val	Thr	Ser		Thr	Asn	Met	Cvs		Val	Ile	Asp	Glv	Asn	Ser
249				420					425					430		
	Thr	Thr	Dha		Uic	Gln	Gln	Pro		Gln	Aen	Δνα	Mot		T.vs	Pho
251	* 11T	T 11T	435	01u	1113	O T 11	0111	440	Leu	11.20	,.sp	**** 9	445	1 110		
	C111	T 013		7 r~	λνα	Lou	λαν	His	λου	Dho	G1 17	Lare		ጥኮው	T.370	Gln
	GIU	450	T 11T	Arg	ату	шeu	455	HITS	vah	FIIG	вту	460	Val	1111	пуз	OIII
253	C1		T	7 ~~	Dha	nha		m~~	7 J ~	T ***	7. c.~		W= 1	17 n 1	C1.,	Wa I
		VdТ	гÀг	ASP	File		Arg	Trp	ATG	пλг		uis	val	val	GIU	
	465	TT 2 -	~1	nk -	m	470	T ~	T	C1	C1	475	T ~	T ***	λ	Dwo	480
	GIU	HIS	GIU	File		vdl	ьys	Lys	етА		HIG	пÄр	пλг	Arg		WIG
257					485					490					495	

VERIFICATION SUMMARY

PATENT APPLICATION: US/10/022,249 TIME: 14:19:14

DATE: 02/05/2002

Input Set : N:\jumbos\022249
Output Set: N:\CRF3\02052002\J022249.raw

 $\ \ \ L:11 \ \ M:270 \ \ C: \ \ Current \ \ Application \ \ Number \ \ differs, \ \ Replaced \ \ Current \ \ Application \ \ Number \ \ L:12 \ \ M:271 \ \ C: \ \ Current \ \ Filing \ \ Date \ \ differs, \ \ Replaced \ \ Current \ \ Filing \ \ Date \ \ \ \$